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METHOD DEVELOPMENT AND VALIDATION OF ITRACONAZOLE BY UV-SPECTROPHOTOMETER

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ABSTRACT

Itraconazole is a synthetic triazole antifungal agent. Itraconazole was formulated into several pharmaceutical forms through various routes of administration. Itraconazole capsules are used to treat fungal infections in the lungs that can spread throughout the body. For quality control and stability testing of Itraconazole in pharmaceutical formulations, limited methods have been published, because the drug is not yet official in any pharmacopoeia. The present work is have made an attempt to develop a more precise, simple and economical spectrophotometric method with greater precision, accuracy and sensitivity for the analysis of Itraconazole in bulk and capsule dosage forms. UV spectroscopic determination was carried out at an absorption maximum of 267 nm using Chloroform as solvent. In the UV spectroscopic method linearity over the concentration range of Itraconazole was found to be 1-10 µg/ml with a correlation coefficient 0.999. Results of the analyses were validated statistically and by recovery studies. The method was validated by the parameters like linearity, precision, accuracy, limit of detection and limit of quantification were studied according to International Conference on Harmonization guidelines.

KEY WORDS: Itraconazole, pharmacopoeia, UV spectroscopic method, Quality control.

INTRODUCTION

Itraconazole is a synthetic triazole antifungal agent. Itraconazole is a 1:1:1:1 racemic mixture

of four diastereomers (two enantiomeric pairs), each possessing three chiral centers. It may be represented by the following nomenclature: 4-[4-[4-[4-[[2-(2, 4-dichlorophenyl)- 2- (1H-1, 2, 4- triazol- 1-ylmethyl)- 1,3- dioxolan- 4- yl] methoxy]phenyl] piperazin-1- yl]phenyl]-2-(1-methylpropyl)-2, 4-dihydro-1, 2, 4-triazol- 3-one (Fig 1). It has a molecular formula is $C_{35}H_{38}C_{l2}N_8O_4$ and a molecular weight is 705.64. [1-4] It is a white to slightly yellowish powder. It is very slightly soluble in alcohols, and freely soluble in dichloromethane. Itraconazole is highly lipophilic in nature and practically insoluble in water. It is an extremely weak base (pKa =3.7) that is ionized only at very low pH. It is a hydrophobic anti mycotic drug with three chiral centers and is used clinically as a stereo isomeric mixture. [5] It is an orally active triazole antifungal agent, which demonstrates broad spectrum activity against a number of fungal species including dermatophytes, Malassesia further, Candida species, Aspergillus species, and Histoplasma capsulatum var. capsulatum. [6,7] The mechanism of action of itraconazole is it interacts with 14-α demethylase, a cytochrome P-450 enzyme necessary to convert lanosterol to ergosterol. As ergosterol is an essential component of the fungal cell membrane, inhibition of its synthesis results in increased cellular permeability causing leakage of cellular contents. Itraconazole may also inhibit endogenous respiration, interact with membrane phospholipids, inhibit the transformation of yeasts to mycelial forms, inhibit purine uptake, and impair triglyceride and/or phospholipid biosynthesis. Itraconazole is metabolized predominately by the cytochrome P450 3A4 isoenzyme system (CYP3A4) in the liver, resulting in the formation of several metabolites, including hydroxyl itraconazole, the major metabolite. [8] Its oral bioavailability was found to increase when taken with food, with plasma concentration approximately two times that taken in the fasting state. [9] It is extensively metabolized in the liver, mainly via an oxidative pathway, into the bioactive metabolite hydroxyl itraconazole. [10]

Itraconazole was formulated into several pharmaceutical forms through various routes of administration. Itraconazole capsules are used to treat fungal infections in the lungs that can spread throughout the body. Used to treat fungal infections of the fingernails. Tablets and capsules are used to treat fungal infections of the toenails. Itraconazole oral solution (liquid) is used to treat yeast infections of the mouth and throat or of the esophagus (tube that connects the throat to the stomach) [11] For quality control and stability testing of Itraconazole in pharmaceutical formulations, limited methods have been published, because the drug is not yet official in any pharmacopoeia. Several HPLC [12], and LC/MS-MS [13-15] methods have been reported for the analysis of Itraconazole in plasma that suffer from either undesirably

long chromatographic run times and requirement for gradient analysis or use of an internal standard one spectrophotometric method [16] have also been reported Spectroflourimetry method has been used for assay of Itraconazole in raw material and in dosage forms. RP-HPLC method is used for determination of Itraconazole in human plasma. [17-21] Chromatographic separation in this method was performed on an octadecyl silane column using fluorescence detector. However, it has the disadvantage of being time consuming. All these studies have further emphasized the need to perform rapid and sensitive quality-control analysis of pharmaceutical formulations containing Itraconazole. As these methods are expensive, we have made an attempt to develop a more precise, simple and economical spectrophotometric method with greater precision, accuracy and sensitivity for the analysis of Itraconazole in bulk and dosage forms.

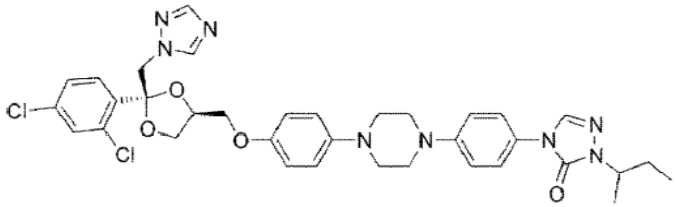


Figure 1. Chemical Structure of Itraconazole

Experimental

Chemicals and reagents: Chloroform was used throughout UV spectrophotometric method development and validation.

Instrumentation

UV spectrophotometric method was performed on double beam UV-visible spectrophotometer (Shimadzu, model 1700) having two matched quartz cells with 1 cm light path.

Selection of solvent

Chloroform was selected as ideal solvent for spectrophotometric analysis of Itraconazole

PREPARATION OF STANDARD STOCK SOLUTIONS

Accurately weighed quantity of 20 mg Itraconazole reference standard was transferred into 20 ml volumetric flask and dissolved and diluted up to the mark with chloroform to give a stock solution having strength 1000µg/ml. 100 µg/ml working standard solution was prepared by diluting 1 ml of stock solution to 10 ml with chloroform.

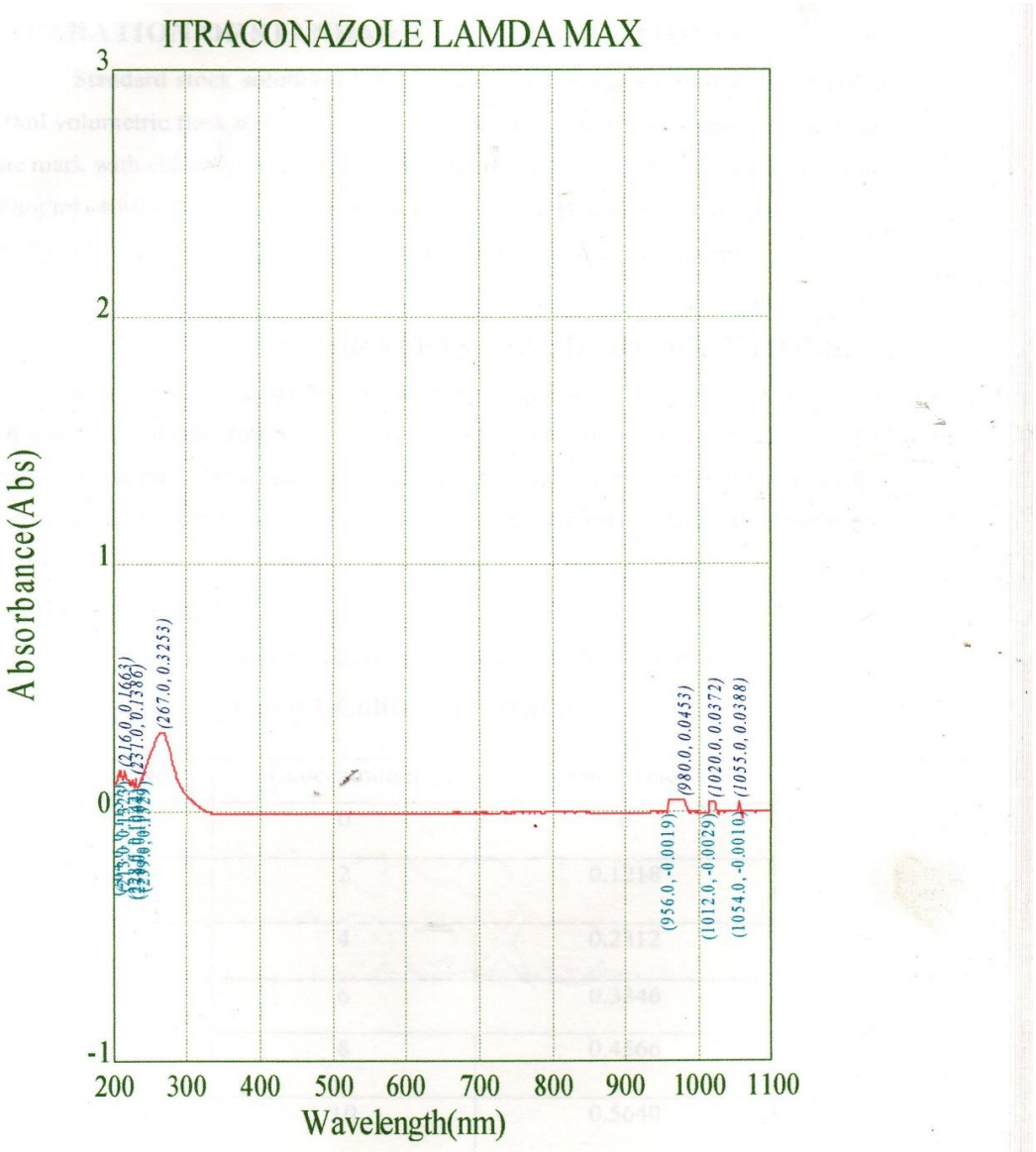
Preparation of Sample stock solution

For analysis of drug in capsule dosage form, 10 capsules were weighed accurately and the powder triturated in the mortar to get a fine powder. The capsule powder equivalent to 10mg was weighed and transferred to 10ml volumetric flask and dissolved with chloroform. The capsule solution was diluted to get a final concentration of 10µg/ml. The absorbance of these solutions measured at 267 nm. The amount of Itraconazole per capsule was calculated using the calibration curve. The readings are shown in table.1

Formula: %Purity=Sample absorbance / Standard absorbance X 100

Table 1: Assay of Itraconazole Capsule.

Brand Name	Lable Claim	Amount prepared	% Purity
Sporanox	100mg	10µg/ml	99.54



METHOD VALIDATION

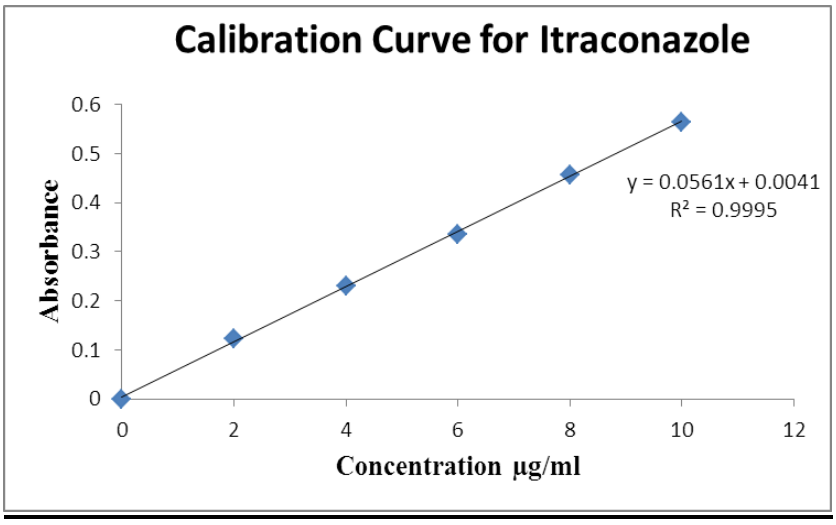
The method was validated according to International conference on Harmonization (ICH) Q2B guidelines 1996 for validation of analytical procedure in order to determine the linearity, limit of detection, accuracy and precision.

LINEARITY & RANGE

Under the experimental conditions, the calibration graphs of the absorbance versus concentration were found to be linear over the range of 0.2-1.0µg/ml for proposed method. The statistical analysis of data obtained for estimation of Itraconazole is indicated chloroform of accuracy for the proposed methods evidenced by the low values of standard deviation and coefficient of variation. The results are shown in table.2

Table 2: Linearity Data of Itraconazole.

S. No.	Concentration µg/ml	Absorbance
1	0	0
2	2	0.1316
3	4	0.2419
4	6	0.3516
5	8	0.4615
6	10	0.5612
Slope:0.056 Regression:0.999		



ACCURACY

To determine the accuracy of proposed method, recovery studies were carried out by adding different amounts (50%, 100%, 150%) of standard bulk sample of Itraconazole within the linearity range were taken and add to pre analyzed formulation of concentration 10µg and

percentage recovery values are calculated . Test should be prepared in triplets at each spike level and assay should be done as per the test method. The values are shown in the table 3.

Table 3: Accuracy & % Recovery Reading.

S. No	Amount Of Sample (µg)	Amount of Standard Added(µg)	Total Amount of Itraconazole	Amount of Itraconazole found	% Recovery	% Mean Recovery	S.D	%RSD
50%	10	5	15	14.95	99.6%	99.7%	0.0011	0.183
50%	10	5	15	14.98	99.8%			
50%	10	5	15	14.96	99.7%			
100%	10	10	20	20	100%	100%	0.0057	0.106
100%	10	10	20	20.25	101%			
100%	10	10	20	20	100%			
150%	10	15	25	24.7	99.1%	99.5%	0.0040	0.403
150%	10	15	25	24.9	99.6%			
150%	10	15	25	25	100%			

PRECISION

The precision of a method is defined as the closeness of agreement between independent test results obtained under optimum conditions. The precision of the ascertained by determination of six replicates of same concentration of sample and standard for method precision and system precision.

SYSTEM PRECISION

The system precision of the proposed method was ascertained by determination of six replicates of same concentration of standard drug within the Beer's range and finding out the absorbance. The absorbance, standard deviation, and %RSD were calculated. Results are shown in the table 4.

Table 4: System Precision Readings.

S. No	Concentration µg/ml	Absorbance
1	10	0.5440
2	10	0.5438
3	10	0.5432
4	10	0.5428
5	10	0.5424
6	10	0.5420
Standard Deviation		0.00115
%RSD		0.2761

METHOD PRECISION

The method Precision of the proposed method was ascertained by determination of six replicates of same concentration of sample drug within the Beer's range and finding out the absorbance. The absorbance, standard deviation and % RSD were calculated. Results are shown in the table.5

Table 5: Method Precision Readings.

S. No	Concentration µg/ml	Absorbance
1	10	0.5390
2	10	0.5388
3	10	0.5385
4	10	0.5380
5	10	0.5378
6	10	0.5374
Standard Deviation		0.000619
%RSD		0.1149

LIMIT OF DETECTION AND LIMIT OF QUANTITATION

The detection limit of individual analytic procedure is the lowest amount of analyte in a sample which can be detected but not necessarily quantitated as an exact value. The quantification limit of an individual analytic procedure is the lowest amount of analyte in a sample which can be quantitatively determined with suitable precision and accuracy. The LOD and LOQ were calculated by using the relation 3.3 σ/S and 10 σ/S respectively, where σ is the standard error of estimate and S is the slope. Calculated values of LOD and LOQ for Itraconazole were found to be 0.14 µg/ml and 0.43 µg/ml respectively. The results are shown in table no: 6

Table 6: Limit of Detection and Limit of Quantitation.

S. No	Conc. (µg/ml)	Absorbance	Standard Deviation	Slope	Limit of Detection	Limit of Quantification
1	ITRACONAZOLE 10 µg/ml	0.5440	0.00115	0.051	0.2	0.5
2		0.5438				
3		0.5432				
4		0.5428				
5		0.5424				

RUGGEDNESS

The ruggedness of the proposed method was evaluated by applying the developed procedures to assay of 10µg/ml of Itraconazole using the same instrument by two different analysts under

the same optimized conditions at different days. The obtained results were found to reproducible, since there was no significant difference between analysts. Thus, the proposed method could be considered rugged. The results are shown in table.7

TABLE 7: RUGGEDNESS

S. No	Analysts	Conc. (µg/ml)	Absorbance	Standard Deviation	%RSD
1	Analyst-I	10	0.5390	0.000619	0.1149
		10	0.5388		
		10	0.5385		
		10	0.5380		
		10	0.5378		
		10	0.5374		
2	Analyst-II	10	0.4882	0.000757	0.1547
		10	0.4886		
		10	0.4892		
		10	0.4897		
		10	0.4899		
		10	0.4901		

ROBUSTNESS

The robustness of the method was determined by introducing small changes in UV parameters, such as changing in the wavelength ± 4. The results are shown in table.8

TABLE 8: RESULTS OF ROBUSTNESS

S. No	Wavelength(nm)	Absorbance
1	267	0.5280
2	271	0.5288
3	263	0.5272

ANALYSIS OF PHARMACEUTICAL FORMULATIONS

The optimized spectrophotometric method applied to the direct determination of Itraconazole in tablet using calibration curve method without any sample extraction or filtration. From the absorbance value, the drug content per tablet was calculated. The results are shown in table.9

Table 9: Analysis of Pharmaceutical Formulations.

Formulation	Labeled Amount (mg)	Amount Recovered (mg)	% Drug Recovered	Mean	Standard Deviation	%RSD
Sporanox	100	93.26	98.315	99.838	1.9398	1.9430
Sporanox	100	102.09	102.022			
Sporanox	100	96.71	99.177			

RESULTS AND DISCUSSION

The goal of the study is to validate an Itraconazole in dosage form by UV-Spectrophotometric method it was carried out under optimized conditions. It was validated the result of the validation parameters were within acceptable limits. Itraconazole follow linearity within the concentration range of 1-10µg/ml. The observed linearity range fitted well Beer-Lambert's law and corresponding regression coefficient (r=0.999) is an indicating of a high degree of method sensitivity the results were tabulated in Table.2.The percentage of the drug found in formulations and results of analysis shows that the amount of drug was in good agreement with the label claim of the formulation. The % RSD is less than to which shows that the system and method has good reproducibility. The results were tabulated. The percentage recovery values of pure drug from the analysed solution of formulation were in between 98-102 which indicated the proposed method were accurate. The results were tabulated in table.3.The validation of proposed method was verified by system precision and method precision the % RSD for system precision of Itraconazole was 0.5 and the data was tabulated in table.4. The method precision was conducted and the percentage average obtained for Itraconazole is shown in table.6.The results obtained from robustness studies i.e., on verified by changing parameters such as wavelength indicated that the analytical method remains unaffected. The study ruggedness made by conducting different systems and by two analysts the results was shows in table.8.The proposed method for Itraconazole in capsules were sample, precise, accurate, rapid and sensitive.

SUMMARY AND CONCLUSION

The method were found to be rapid, economical, accurate and precise for the determination of Itraconazole in bulk drug in capsule by UV-Spectrophotometer methods produce comparable results can be used for precise and accurate analysis of Itraconazole in its pure and capsule dosage form. Interference studies reviled that the common excipients and other activities usually present in the dose. The values of % recovery was close to 100% indicating reproducibility and accuracy of the proposed method successfully employed as a quality control tool for the analysis of Itraconazole in its capsule dosage form and in bulk drug.

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